

Substitution of Cottonseed Meal for Marine Animal Protein in Diets for *Penaeus vannamei*

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Abstract

Solvent-extracted cottonseed meal was used in shrimp *Penaeus vannamei* diets at levels of 0, 13.3, 26.5, 39.8, 53.0 and 66.3%, substituting on an equal nitrogen basis for 0, 20, 40, 60, 80 and 100% of animal protein mix (53% menhaden fish meal, 34% shrimp waste meal and 13% squid meal). The feeds were formulated to contain 32% crude protein and 3,100 kcal metabolizable energy/kg. Each diet was fed to juvenile shrimp to satiation four times daily for 8 wk. Shrimp fed the three lowest dietary levels of cottonseed meal (0, 13.3 and 26.5%) had similar weight gain, feed consumption and survival. The performance of shrimp was adversely affected when diets containing more than 26.5% cottonseed meal, or 1,100 ppm free gossypol, were fed. Shrimp fed the diet with 39.8% cottonseed meal or 1,600 ppm free gossypol had depressed weight gain, reduced feed intake and high mortality. The groups receiving the two highest dietary levels of cottonseed meal lost weight by the end of week 4 and all shrimp in these treatments died within 6 to 8 wk. These adverse effects were probably due to the toxicity of free gossypol. Shrimp appeared to accumulate gossypol in the body as evidenced by light yellow-green coloration in shrimp fed diets containing cottonseed meal.

Feed is normally the single largest expenditure in semi-intensive and intensive shrimp culture operations, and protein is the most expensive component in feeds for shrimp. This is due to extensive reliance on marine animal protein such as fish meal, shrimp meal and squid meal to meet the high dietary protein requirements for shrimp. These feedstuffs have high nutritional value and palatability but are expensive and not always readily available (Lim and Dominy 1990). Thus, replacement of these high-cost ingredients with less expensive plant proteins would be beneficial in reducing feed costs. Cottonseed meal, which ranks second in the United States (Dorsa et al. 1982) and third in the world (Swick and Tan 1995) in tonnage among the vegetable protein concentrates produced, is available at a much lower cost than animal proteins. At present, cottonseed meal is widely used as a protein supplement for ruminant livestock but its use in non-ruminant animal feeds is limited due in part to the presence of gossypol, a toxic component for monogastric animals (Swick and

Tan 1995), and the low concentration of lysine.

The nutritional value of cottonseed meal has been evaluated for several species of finfish such as chinook and coho salmon (Fowler 1980), tilapia (Jackson et al. 1982; Ofojekwu and Ejike 1984; Robinson et al. 1984; Viola and Zohar 1984; El-Sayed 1990) and channel catfish (Dorsa et al. 1982; Robinson and Rawles 1983; Robinson et al. 1983; Robinson and Daniels 1987; Robinson and Brent 1989; Robinson 1991; Robinson and Li 1994; Robinson and Tiersch 1995). Generally, it has been reported that the amount of cottonseed meal that can be included in fish feeds depends on the species and levels of free gossypol, dietary protein and available lysine.

Fernandez and Lawrence (1988) reported that different levels of cottonseed meal can be incorporated in the diets of penaeid shrimp depending on the shrimp species and dietary protein levels. Cottonseed meal can also be used as a substitute for fish and shrimp head meals in shrimp diets without reducing growth, but at lower levels than

TABLE 1. *Percentage composition of experimental diets.*

Ingredient	Diet number					
	1	2	3	4	5	6
Animal protein mix ^a	48.00	38.40	28.80	19.20	9.60	—
Cottonseed meal	—	13.30	26.50	39.80	53.00	66.30
Whole wheat flour	10.50	10.50	10.50	10.50	10.50	10.50
Corn starch	17.76	14.20	10.66	7.10	3.55	—
Wheat gluten	4.00	4.00	4.00	4.00	4.00	4.00
Soybean lecithin	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50
Fish oil	—	0.84	1.67	2.51	3.34	4.20
Cottonseed oil	1.76	1.41	1.06	0.70	0.35	—
Calcium carbonate	—	1.02	2.04	3.06	4.08	5.10
Dicalcium phosphate	0.65	1.35	2.10	2.82	3.56	4.29
Dipotassium phosphate	2.34	2.12	1.85	1.60	1.36	1.11
Vitamin mix ^b	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix ^c	1.00	1.00	1.00	1.00	1.00	1.00
Celufil	6.39	5.10	3.83	2.56	1.28	—
Diatomaceous earth	4.10	3.26	2.49	1.65	0.88	—
Percent animal protein mix replaced by cottonseed meal	0	20	40	60	80	100

^a Mixture consisting of 53% menhaden fish meal, 34% shrimp residue meal and 13% squid meal.

^b Vitamin mixture providing the following vitamins (mg/kg diet): vitamin A, 80,000 IU; vitamin D₃, 2,000 IU; vitamin E, 200 IU; vitamin K, 20; thiamin hydrochloride, 60; riboflavin hydrochloride, 60; pyridoxine, 100; d-calcium pantothenate, 150; niacin, 300; biotin, 2; folic acid, 20; cyanocobalamine, 0.1; ascorbic acid, 600; choline chloride, 3,000.

^c Modified BT_m salt mixture plus the following minerals (mg/kg diet): zinc, 31.5; iodine, 4.8; selenium, 0.4; cobalt, 0.4.

soybean meal (Lawrence and Castille 1989).

No studies, however, have yet examined cottonseed meal as a substitute for a mixture of fish, shrimp and squid meals in shrimp diets. Thus, this study was conducted to compare the growth, survival, feed conversion, protein efficiency ratio and body composition of juvenile *Penaeus vannamei* fed diets containing different levels of cottonseed meal as a substitute for a marine animal protein mix.

Materials and Methods

Six practical-type diets were prepared to contain 0, 13.3, 26.5, 39.8, 53.0 and 66.3% solvent-extracted cottonseed meal (0.41% free gossypol) as replacements of 0, 20, 40, 60, 80 and 100% of a marine protein mix consisting of 53% menhaden fish meal, 34% shrimp residue meal and 13% squid meal (Table 1). All diets were formulated

to contain approximately 32% crude protein and 3,100 kcal/kg metabolizable energy (ME). Since the energy values of various feedstuffs are not available for shrimp, ME values were calculated on physiological values of 4 kcal/g for protein and carbohydrate (excluding crude fiber) and 9 kcal/g for lipid (Maynard and Loosli 1979). The dietary level of oil was maintained constant by the addition of menhaden fish oil and cottonseed oil. Other dietary components such as cholesterol, lecithin, total phosphorus and potassium were the same in all diets. Celufil and corn starch were used to adjust the dietary level of crude fiber and nitrogen-free extract, respectively. Proximate composition and free gossypol content of the experimental diets are given in Table 2.

All ingredients were ground to pass through a 0.75-mm mesh screen and processed into 3-mm diameter pellets. The diets were prepared and stored as described

TABLE 2. Proximate composition and estimated gossypol content of experimental diets.

Diet number	Nutrient (% on as fed basis)					Free gossypol (% of diet) ^a
	Moisture	Crude fat	Crude protein	Crude fiber	Ash	
1	6.45	7.33	32.50	7.73	17.92	0.00
2	7.27	7.38	32.23	9.09	17.10	0.05
3	8.09	7.38	31.94	8.45	15.95	0.11
4	8.14	7.29	32.14	9.25	15.33	0.16
5	9.36	7.53	31.79	10.19	14.36	0.22
6	9.57	6.70	31.99	10.81	13.65	0.27

^a Calculated from the free gossypol content of cottonseed meal.

by Lim and Dominy (1990) except that drying was done in a Hobart oven at 75 C for 25 min.

Juvenile *Penaeus vannamei* obtained from a commercial shrimp farm (Amorient Aquafarm Inc., Kahuku, Hawaii, USA) were acclimated to laboratory conditions for two weeks and fed a commercial feed twice daily. After acclimatization, shrimp ranging from 0.77 to 1.39 g (average 1.03 \pm 0.18 g) were weighed and stocked into 24 flow-through 60-L glass aquaria at a density of 15 shrimp/aquarium. Four aquaria, arranged in a completely randomized design, were assigned to each of the experimental diets. Each aquarium, filled with 52 L of seawater, was provided with a tight-fitting netting cover, plastic netting shelter and continuous aeration with an air stone. Shrimp that died within 24 h after stocking were replaced by shrimp of similar size.

Each test diet was fed to shrimp four times daily to satiation for 8 wk. Feeds were offered two times in the morning between 0830 and 0930 h and two times in the afternoon between 1500 and 1600 h. The amount of feed consumed per aquarium was determined daily.

All aquaria were cleaned daily by siphoning off accumulated waste materials and exuviae. Water flow rates were checked and adjusted daily to insure proper water exchange rate. Photoperiod was maintained on a 12:12 h light:dark schedule. Water temperature, salinity, dissolved oxygen and pH were measured in three randomly se-

lected aquaria three times per week. Water temperature ranged from 25.8 to 26.8 C with an average of 26.5 \pm 0.2 C; salinity varied from 35.0 to 37.0 ppt and averaged 35.8 \pm 0.6 ppt; dissolved oxygen ranged from 5.9 to 6.3 ppm with an average of 6.1 \pm 0.1 ppm; pH value fluctuated from 8.0 to 8.5 and averaged 8.2 \pm 0.1.

Every 2 wk, the shrimp in each aquarium were counted and weighed *en masse*. When the shrimp were sampled, each aquarium was thoroughly cleaned, drained and re-filled. On sampling days, shrimp were fed once in the afternoon with 60% the amount of feed consumed the previous day to minimize cannibalism. All experimental diets were analyzed in triplicate for proximate composition by a private laboratory using accepted procedures published in the literature. Free gossypol content of cottonseed meal was analyzed by the Mississippi State Chemical Laboratory, Mississippi State University, Mississippi State, Mississippi, USA.

At the conclusion of the experiment, all shrimp were collected and stored frozen at -8 C for subsequent determination of whole body composition. Proximate analyses of shrimp were done in triplicate by Woodsen Tenent Laboratories, Inc., Memphis, Tennessee, USA.

Feed conversion ratio (FCR) was determined as the grams of dry weight of feed fed per gram of wet weight gain. The protein efficiency ratio (PER) was calculated

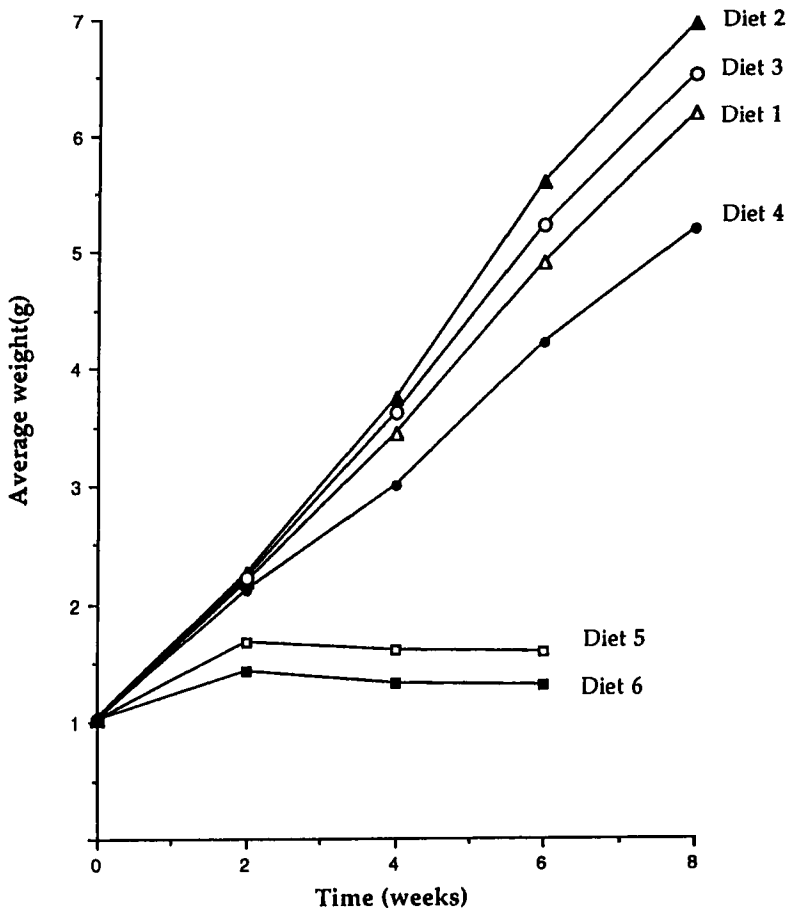


FIGURE 1. Average weight of *P. vannamei* fed diets containing various levels of cottonseed meal.

as the grams of wet weight gain per gram of crude protein fed.

All data were subjected to one-way analysis of variance and Duncan's multiple-range test (SAS Institute Inc. 1993). Differences were considered significant at the 0.05 probability level.

Results

Average body weights of shrimp at 2 wk intervals are presented in Fig. 1. The growth response and feed utilization efficiency are given in Table 3. Shrimp fed diets containing 0, 13.3 and 26.5% of cottonseed meal (diets 1, 2 and 3) exhibited similar growth patterns throughout the 8-wk period, although shrimp fed diet 2 were heavier than those fed diet 1 and 3. No sig-

nificant differences, however, were observed among the final weight gain of shrimp in these treatments. Growth rate declined when the dietary level of cottonseed meal was increased to 39.8% or greater (diets 4, 5 and 6). By the end of week 4, the weight gain of shrimp fed diet 4 was significantly lower than those of shrimp fed diets 1, 2 and 3. The groups fed diets 5 and 6 weighed significantly less than those of the other treatments at the end of week 2, and lost weight at weeks 4 and 6.

Shrimp fed diet 3 had the highest survival rate but was not significantly different from those fed diets 1 and 2. Shrimp fed diet 4 had significantly lower survival than those of shrimp fed diets 1, 2 and 3. Mortality for shrimp receiving the two highest

TABLE 3. Mean (\pm SE) weight gain, survival, dry matter feed intake, feed conversion and protein efficiency for shrimp fed diets containing various levels of cottonseed meal for 8 wk. Values in the same column having the same superscript are not significantly different ($P > 0.05$).

Diet number	Weight gain (g)	Survival (%)	Dry matter feed intake (g/shrimp)	Feed conversion ratio ¹	Protein efficiency ratio ²
1	5.15 \pm 0.33 ^a	91.67 \pm 3.19 ^a	8.41 \pm 0.24 ^a	1.65 \pm 0.08	1.76 \pm 0.08
2	5.90 \pm 0.12 ^a	93.34 \pm 3.85 ^a	9.01 \pm 0.15 ^a	1.52 \pm 0.04	1.89 \pm 0.05
3	5.47 \pm 0.15 ^a	96.67 \pm 3.33 ^a	8.90 \pm 0.11 ^a	1.63 \pm 0.05	1.77 \pm 0.05
4	4.15 \pm 0.40 ^b	50.00 \pm 15.99 ^b	4.87 \pm 1.71 ^b	1.74 \pm 0.05	1.65 \pm 0.11
5 ³	0.57 \pm 0.13	10.00 \pm 3.33	—	—	—
6 ³	0.28 \pm 0.20	10.00 \pm 5.77	—	—	—

¹ Feed conversion = Dry feed fed (g)/wet weight gain (g).

² Protein efficiency ratio = Wet weight gain (g)/protein consumed (g).

³ All shrimp died between week 6 to 8. Values presented are data at week 6.

levels of cottonseed meal (diets 5 and 6) began after the second week and only 10% of shrimp in each of these treatments survived by the end of week 6. All shrimp fed these diets died between weeks 6 and 8.

Total feed intake data (dry matter basis) reflected the weight gain data. Dry matter feed intake was highest for shrimp fed diet 1 and was followed by those fed diets 3 and 1. No significant differences were found among the feed intakes of shrimp receiving diets 1, 2 and 3. Feed consumption significantly decreased when cottonseed meal levels were increased to 39.8% or higher.

Feed conversion ratio and protein efficiency ratio were also a reflection of the weight gain, with shrimp fed diet 2 having the best FCR and PER. No significant differences were observed among the values for diets 1, 2, 3 and 4.

Whole body composition of shrimp ex-

pressed as percent dry matter is given in Table 4. Percentages of moisture, crude protein, crude fat and ash significantly differed among shrimp fed different diets. Moisture content was highest for shrimp fed diet 4 but varied only slightly for those fed diets 1, 2 and 3. Percentage of protein ranged from 76.43% for shrimp fed diet 1 to 77.89% highest for shrimp fed diet 4. Body fat was highest for shrimp fed diet 2 and lowest for those fed diet 4. Ash content followed the reverse trend of that of body fat.

Discussion

The present study showed that 26.5% solvent-extracted, glanded cottonseed meal (0.41% free gossypol) can be used to replace 40% of an animal protein mix in the diet of juvenile *Penaeus vannamei* without affecting the overall performance. Previous studies have shown that the ability of

TABLE 4. Whole body percentage composition of shrimp fed diets containing various levels of cottonseed meal for 8 wk. Values in the same column having the same superscript are not significantly different ($P > 0.05$).

Diet number	Nutrient (% dry matter)			
	Moisture	Crude protein	Crude fat	Ash
1	74.07 \pm 0.05 ^c	76.43 \pm 0.01 ^d	4.33 \pm 0.01 ^c	13.09 \pm 0.03 ^b
2	74.35 \pm 0.09 ^b	76.75 \pm 0.01 ^c	6.87 \pm 0.01 ^a	12.48 \pm 0.01 ^d
3	74.17 \pm 0.09 ^{ab}	77.53 \pm 0.04 ^b	5.74 \pm 0.03 ^b	12.64 \pm 0.01 ^c
4	76.42 \pm 0.04 ^a	77.89 \pm 0.01 ^a	3.90 \pm 0.02 ^d	13.88 \pm 0.03 ^a
5 ¹	—	—	—	—
6 ¹	—	—	—	—

¹ All shrimp died between week 6 to 8.

shrimp and fish to utilize cottonseed meal varies depending on the species, level of free gossypol and composition of the diets, particularly as it relates to the quality and quantity of protein. Fernandez and Lawrence (1988) reported that weight gain and survival of postlarval *P. vannamei* and *P. stylirostris* were not affected when 20% cottonseed meal was included in a 30% protein diet. However, significant growth depression was observed when the same diet was fed to *P. setiferus*. For feeds containing 20% protein, up to 10% cottonseed meal could be added without affecting growth of *P. vannamei* and *P. stylirostris*. Adding 5% or higher levels of cottonseed meal to 20% protein diets for *P. setiferus* resulted in a significant decrease in weight gain. Lawrence and Castille (1989) indicated that cottonseed meal can replace shrimp head and fish meals in penaeid shrimp diets without affecting growth, but at lower levels than soybean meal. Fowler (1980) showed that cottonseed meal was effectively utilized as a replacement of fish meal in diets of chinook salmon and coho salmon up to levels of 34.1% and 22.0%, respectively. Jackson et al. (1982) obtained good growth of tilapia *Sarotherodon mossambicus* when 35.2% prepressed solvent-extracted cottonseed meal (0.03% free gossypol) was used as a substitute for 50% of fish meal. The growth rate was essentially the same as the control even at 100% substitution level. Likewise, El-Sayed (1990) demonstrated that cottonseed meal can be used as a main dietary protein source for *O. niloticus*. Viola and Zohar (1984) found that low-gossypol cottonseed meal can be included in feeds for hybrid tilapia *O. niloticus* × *O. aureus* to the same extent as soybean meal. In contrast, Robinson et al. (1984) reported that neither glanded nor glandless cottonseed meals appeared to be as high in nutritive value for *Tilapia aurea* as soybean and peanut meals. Ofojekwu and Ejike (1984) also reported a much lower weight gain and feed efficiency of *O. niloticus* fed a cottonseed cake diet as compared to tilapia fed

the fish meal control diet. For channel catfish, Dorsa et al. (1982) found that 17.4% cottonseed meal (0.49% free gossypol) could be included in the diets without adverse effects on growth. Robinson and Daniels (1987) recommended that glanded cottonseed meal should not be used in channel catfish feeds at levels higher than 15% of the diet. However, with low-gossypol cottonseed meal (0.022% free gossypol) a level of up to 25–30% could be used in channel catfish feeds without detrimental effects (Robinson 1991). He suggested that if higher levels of cottonseed meal are to be used in channel catfish feeds, protein quality must be improved by supplementation of lysine.

Although shrimp can tolerate a dietary level of up to 26.8% cottonseed meal or 1,100 ppm free gossypol, shrimp fed diets containing cottonseed meal developed light yellow-green coloration at the end of week 4. The color intensity increased with increasing levels of dietary cottonseed meal. Morrison (1961) reported that if the ration of laying hens contain more than 5% of ordinary cottonseed meal, the yolks of the eggs are apt to develop an olive green or brown color and the albumen a pinkish color on storage. In contrast, Dorsa et al. (1982) did not observe any yellowing or discoloration of the muscle of channel catfish fed diet with up to 29% cottonseed meal or 1,400 ppm free gossypol. However, they found that whole body level of free gossypol increased for fish fed higher amounts of cottonseed meal, although free gossypol was most concentrated in liver and kidney. Robinson and Daniels (1987) also reported that accumulation of free gossypol in muscle tissue was not a problem in channel catfish fed 23.3% glanded or 52.8% glandless cottonseed meals for 28 wk.

Free gossypol, when present in sufficient quantity in the diet, has been shown to be toxic to monogastric animals. General symptoms of gossypol toxicity are anorexia, poor growth and high mortality (Morri-

son 1961; Roehm et al. 1967; Berardi and Goldblatt 1980). Thus, growth depression, decreased feed intake and heavy mortality observed in shrimp fed diets containing more than 26.8% cottonseed meal or 1,100 ppm free gossypol was probably due to the direct toxic effects of gossypol. The growth of channel catfish was inhibited when fed diets with more than 17.4% cottonseed meal or 900 ppm free gossypol (Dorsa et al. 1982). Herman (1970) reported that a dietary concentration of 300 ppm free gossypol was toxic to rainbow trout. *Tilapia aurea*, however, can tolerate a relatively high level of free gossypol. Dietary levels of purified gossypol up to 1,800 ppm did not affect growth, feed efficiency or survival of this species (Robinson et al. 1984).

The presence of free gossypol also decreases the protein quality of cottonseed meal by binding the epsilon amino group of lysine during heat processing, thus reducing the bioavailability of lysine (Kuiken 1952; Berardi and Goldblatt 1980). The true lysine availability of cottonseed meal for channel catfish has been reported to be about 71% (Wilson et al. 1981). Robinson and Li (1995) indicated that the toxicity of gossypol for channel catfish appears to be less of a problem than lysine deficiency. The latter, however, can be remedied by lysine supplementation. The detrimental effects observed in our study for shrimp fed dietary cottonseed meal higher than 26.5% was probably not related to lysine deficiency since diet 4 which has 39.8% cottonseed meal still contains about 4.7% (percent of dietary protein) lysine. This value is within the range of 3.7% to 5.7% dietary lysine reported to be optimum for various finfish species (NRC 1993).

Percentage of body fat seemed to be directly related to the weight gain and inversely related to the moisture content. On the other hand, percentage of ash appeared to be inversely related to the size of shrimp. Likewise, Lim (1993) reported that body fat of shrimp seemed to be directly related to

the growth rate, whereas ash content was inversely related to the size of shrimp.

Results of this study showed that up to 26.5% glanded cottonseed meal (0.41% free gossypol) can be used as a replacement for marine animal protein mix in the diets of *Penaeus vannamei*. Increasing dietary cottonseed meal above this level depressed weight gain, feed intake and increased mortality. These adverse effects were probably due to gossypol toxicity rather than lysine deficiency.

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